

CLAIMS

The claimed invention is:

1. A method for assaying homocysteine (Hcy) in a sample without chromatographic separation, which method comprises:
 - a) contacting a sample containing or suspected of containing Hcy with a Hcy co-substrate and a Hcy converting enzyme in a Hcy conversion reaction to form a Hcy conversion product and a Hcy co-substrate conversion product, wherein the Hcy co-substrate is S-adenosylmethionine (SAM), the Hcy converting enzyme is a S-adenosylmethionine (SAM)- dependent homocysteine S-methyltransferase, the Hcy conversion product is methionine (Met) and the Hcy co-substrate conversion product is S-adenosyl-L-homocysteine (SAH);
 - b) assessing the Hcy co-substrate conversion product SAH generated in step (a) by contacting the SAH with a SAH hydrolase to generate Hcy from SAM, which is cycled into the Hcy conversion reaction by the SAM-dependent homocysteine S-methyltransferase to form a Hcy co-substrate based enzyme cycling reaction system, and adenosine (Ado), which is assessed to determine the presence, absence and/or amount of the Hcy in the sample.
2. The method of claim 1, wherein the Ado is contacted with an adenosine converting enzyme other than the SAH hydrolase.
3. The method of claim 2, wherein the assessment of the Ado is effected indirectly by assessment of a co-substrate or a reaction product of adenosine conversion by the adenosine converting enzyme.
4. The method of claim 3, wherein the adenosine converting enzyme is an adenosine kinase.

5. The method of claim 3, wherein the adenosine converting enzyme is an adenosine deaminase.
6. The method of claim 1, wherein the sample is a body fluid or a biological tissue.
7. The method of claim 6, wherein the body fluid is selected from the group consisting of urine, blood, plasma, serum, saliva, semen, stool, sputum, cerebral spinal fluid, tears, mucus and amniotic fluid.
8. The method of claim 6, wherein the body fluid is blood.
9. The method of claim 8, wherein the blood sample is further separated into a plasma or serum fraction.
10. The method of claim 1, wherein prior to or concurrently with the contact between the sample and the Hcy co-substrate and the Hcy converting enzyme, oxidized or conjugated Hcy in the sample is converted into reduced Hcy.
11. The method of claim 1, wherein the Ado is assessed without chromatographic separation.
12. The method of claim 1, wherein the SAM is added to the sample.
13. The method of claim 1, wherein the SAM is produced from ATP and Met by a SAM synthase.
14. A method for assaying homocysteine (Hcy) in a sample, which method comprises:

- a) contacting a sample containing or suspected of containing Hcy with a Hcy co-substrate and a Hcy converting enzyme in a Hcy conversion reaction to form a Hcy conversion product and a Hcy co-substrate conversion product, wherein the Hcy co-substrate is S-adenosylmethionine (SAM), the Hcy converting enzyme is a S-adenosylmethionine (SAM)- dependent homocysteine S-methyltransferase, the Hcy conversion product is methionine (Met) and the Hcy co-substrate conversion product is S-adenosyl-L-homocysteine (SAH),
- b) assessing the Hcy co-substrate conversion product SAH to determine the presence, absence and/or amount of the Hcy in the sample, wherein the SAH is assessed without chromatographic separation.

15. The method of claim 14, wherein the assessment of SAH does not involve an enzymatic reaction generating H₂O₂ and detection of H₂O₂.

16. The method of claim 14, wherein the SAM is added to the sample.

17. The method of claim 14, wherein the SAM is produced from ATP and Met by a SAM synthase.

18. A kit for assaying Hcy in a sample, which kit comprises:

- a) a S-adenosylmethionine (SAM)- dependent homocysteine S-methyltransferase;
- b) S-adenosylmethionine (SAM) or ATP, Met and a SAM synthase;
- c) a SAH hydrolase; and
- d) a reagent for assessing adenosine (Ado).

19. The kit of claim 18, wherein the reagent for assessing Ado comprises an adenosine converting enzyme other than the SAH hydrolase.

20. The kit of claim 18, wherein the adenosine converting enzyme is an adenosine kinase or an adenosine deaminase.

21. A kit for assaying Hcy in a sample, which kit comprises:

- a) a S-adenosylmethionine (SAM)- dependent homocysteine S-methyltransferase;
- b) S-adenosylmethionine (SAM) or ATP, Met and a SAM synthase; and
- c) a reagent for assessing SAH,

wherein the kit does not comprise an enzyme or a reagent for generating H₂O₂ and a reagent for detecting H₂O₂.